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ARS-NC-18
July 1974

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Acknowledgment

The technical assistance of Henry J. Stevens Jr., Forage Insect Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Lincoln, Nebr., in making the many root dissections that were necessary to obtain root borer data is greatfully acknowledged.

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Agricultural Research Service
U.S. DEPARTMENT OF AGRICULTURE
In cooperation with the
Nebraska Agricultural Experiment Station

Resistance in Sweetclover to the Sweetclover Root Borer[1]

by G. R. Manglitz, H. J. Gorz, and F. A. Haskins[2]

The sweetclover root borer, *Walshia miscecolorella* (Chambers), has been reported to be a pest of sweetclover in the Great Plains by Manglitz and others.[3] These authors reported the type of injury to sweetclover, seasonal history observations, and biology notes. All of the sweetclover species, *Melilotus*, observed appeared to serve as hosts for the root borer. This report details concurrent and subsequent observations on differential rates of infestation between and within species of *Melilotus*. These observations indicate that the coumarin content of the plants may be one of the factors responsible for some of the differences in rates of infestation among the various sweetclovers. Although the term "coumarin" is used in this report, in reality, little if any coumarin occurs in the intact sweetclover plant.[4] The plant constituents commonly referred to as coumarin are the glucosides of *cis- and trans-o*-hydroxycinnamic acid.

Materials and Methods

In the initial sampling, several species and varieties of sweetclover were observed for infestations. Subsamples were taken from nonreplicated field-seeded nurseries and from seedlings started in the greenhouse and transplanted to the field in a randomized complete block design with four replications. Resistance was evaluated by dissecting roots and by determining the number of larvae per plant. Percentage of plants infested was calculated and when possible, in the fall, visual damage was scored on a scale of 1 to 5, where 1 = no visual damage to the top growth and 5 = the severest top growth symptoms as described by Manglitz and others.[3]

The effect of coumarin and related compounds on sweetclover root borer infestations was studied using near-isogenic lines of *Melilotus alba* Desr. identical to those used by Gorz and others.[5] that were homozygous for the *Cu/cu* and *B/b* alleles. The independent genes, *Cu/cu* and *B/b*, influence the amount of *o*-hydroxycinnamic acid glucosides and the activity of B-glucosidase, respectively. Plants of the *CuCu* genotype are high in content of *o*-hydroxycinnamic acid glucosides, and preparations of the *BB* genotype possess B-glucosidase activity. The homozygous genotypes were derived from an initial *cucuBB* X *CuCubb* cross.

A single plant, heterozygous for both *Cu/cu* and *B/b*, was selfed in each generation from F_1 to F_{17}. The homozygous genotypes, *CuCuBB*, *CuCubb*, *cucuBB*, and *cucubb*, were isolated in the F_{18} generation and were progeny tested for homozygosity in the F_{19} generation. The F_{21} seed in this study was obtained by selfing in the F_{19} and F_{20} generations. Thus, the four homozygous lines were closely related and highly inbred.

In 1969, observations were made on 20 plants of each genotype taken from a nonreplicated planting. Subsequent observations were made in nurseries containing the four genotypes planted in a randomized complete block design with 12 replication of three plants of each genotype per replication. Evaluation of resistance was made in a manner identical to that described above.

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[1] Agr. Res. Ser., U.S. Dep. of Agr. in cooperation with the Nebr. Agr. Exp. Sta. (Paper No. 3715, J. Ser., Nebr. Agr. Exp. Sta. Contribution No. 377 Dept. Ent., Univ. of Nebr.)

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[3] Manglitz, G. R., H. J. Gorz, and H. J. Stevens, Jr. biology of the sweetclover root borer. J. Econ. Ent. 64: 1154-1158. 1971.

[4] Haskins, F. A. and H. J. Gorz. a reappraisal of the relationship between free and bound coumarin in "melilotus." Crop. Sci. 1: 320-323. 1961.

[5] Gorz, H. J., F. A. Haskins, and G. R. Manglitz. effects of coumarin and related compounds on blister beetle feeding in sweetclover. J. Econ. Ent. 65: 1632-1635. 1972.

Results and Discussion

The initial observations were made in three nonreplicated plots (72 ft. by 18 ft.) of *Melilotus officinalis* var. 'Goldtop', *M. alba* var. 'Denta', and *M. polonica* var. 'PI 314386'. The observations were made in early winter after the top growth had been killed by frost that precluded the scoring of visual damage. Casual scanning of these plots, earlier in the fall of 1967, indicated that the *M. polonica* entry was less heavily infested than the other two. Actual counts of larvae in roots of *M. polonica*, Denta, and Goldtop showed that 36, 66, and 74 percent of the plants of the three species, respectively, were infested, with respective mean counts of 4, 8, and 11 larvae per 10 roots. However, differences among species for percentage of infested plants or number of larvae were statistically nonsignificant.

Differences between 'Goldtop' and 'Denta' had been noted previously,[6] but were not considered great enough to be of practical importance. Thus, the apparent resistance of *M. polonica* was of much greater interest and *polonica* was included in a replicated nursery during 1968 (table 1) along with 'Denta', 'Goldtop', 'Israel' (a variety with annual growth habit), and an entry of *M. taurica*. The *polonica* and *taurica* entries had significantly less infestation than 'Denta' and 'Goldtop', but the former did not look much better when visual scores were compared, presumably because these lines were less tolerant to the root borer than the commercial varieties. *M. polonica* seemed more promising than *M. taurica* because it is more closely related to and can be crossed with *M. alba*.

Annual sweetclovers have been reported as resistant to the root borer[7] although it is possible that the apparent resistance may represent escape because of their earlier maturity. The late-maturing annual variety, Israel, in this study, was heavily infested. The infestation, however, was less severe than for most other entries, probably because roots of annuals are less fleshy and succulent than those of biennials.

During 1969 and 1970, lines resulting from *M. alba* × *M. polonica* crosses were included in the nurseries (table 2). The infestations in 1969 were extremely heavy in the commercial varieties, but resistance in *polonica* and crosses held up well. Unfortunately, an early killing frost prevented the scoring of plant damage. During 1970, infestation rates dropped sharply from the previous year, but the *polonica* crosses again appeared resistant.

The "low coumarin" variety, Denta, reacted in an interesting way under the various infestation levels. Initially, under moderate infestations, Denta had intermediate levels of resistance. Under the heavy infestation of 1969, Denta had no resistance at all, but when the infestation levels were low in 1970, Denta ranked with the most resistant entries. If the resistance of Denta is related to its low coumarin content, possibly coumarin is attractive to the root borer moth and thus Denta is less attractive. When moth populations are high, attractiveness of the host would play less of a role because more insects would discover their hosts by random searching. Funnel traps were baited with coumarin crystals and coumarin-filled vials were fastened to sticky board traps. However, under those conditions, the coumarin-baited devices were no more attractive than the nonbaited devices.

The results with the near isogenic lines (table 3) also suggest that coumarin or its related compounds, or both, are either more attractive to the moths or provide better survival for the larvae

TABLE 1.—Root borer observations on plants of four species of *Melilotus* in the fall of 1968, Lincoln, Nebr.[1]

Entry	Larvae per plant	Percent plants infested[2]	Visual damage score[3]
PI 314094, *M. taurica*	0.87 a	44.5 a	3.0 b
Israel, *M. alba* (annual)	1.60 a	68.3 b	1.8 a
PI 314386, *M. polonica*	2.88 a	80.0 b	5.0 d
Denta, *M. alba*	6.10 b	86.7 b	4.0 c
Goldtop[4], *M. officinalis*	8.48 c	95.0 c	5.0 d
Goldtop[5], *M. officinalis*	13.03 d	98.3 d	5.0 d

[1] Means not followed by the same letter are significantly different from each other—Duncan's Multiple Range Test, 0.05 probability level.

[2] Actual percentages shown. The analysis of variance was based on angle transformations.

[3] Actual rating shown; 1 = no damage, 5 = severe damage. The analysis of variance was based on square root transformations.

[4] Uncertified seed obtained from a commercial source.

[5] Breeder's seed obtained from the Wisconsin Agricultural Experiment Station.

[6] See reference in footnote 3, p. 1.

[7] MORRIS, M. J. NEW INSECT PEST ATTACKS BIENNIAL SWEETCLOVER IN NORTH-CENTRAL TEXAS. Crops Soils 4: 27. 1952.

when the root borer populations are moderate or low. However, during 1971, when the populations were somewhat higher, the resistance seemed to break down as it did in the case of Denta.

Concerning other insects that feed on sweetclover, coumarin may be attractive to sweetclover weevil adults,[8] but it is a strong deterrent to feeding by adult blister beetles.[9] Sweetclover plants carrying the *b* allele are lacking in activity of B-glucosidase, the enzyme that converts "bound coumarin" to the free form.[10] Leaves of *CuCubb* plants appear more attractive to blister beetles than *CuCuBB* leaves, probably because of the potential for free coumarin formation in the latter leaves.[9] The *b* allele did not have a significant effect on sweetclover root borer infestation, which indicates that free coumarin may not be attractive to moths. This unattractiveness explains the negative results obtained in moth trapping when using coumarin crystals as bait. Although, the blister beetles play a more active role than the moths in initiating B-glucosidase activity by chewing leaves, tissue disruption by other means (wind, other insect species, grazing animals, heavy rain, and hail) would also result in such activity even in the absence of blister beetle feeding.

TABLE 2.—Sweetclover root borer infestations in replicated nursery plantings of *M. alba* × *M. polonica* crosses and related entries, Lincoln, Nebr.[1]

Entry	Description of plants	Larvae per plant	Percent infested plants	Visual damage score
1969:				
S 712	*M. taurica*, PI 314,094	3.0 a	86.6 a	—
S 715	*M. alba*, var Israel Annual	3.3 a	90.6 a	—
S 709	F_2 *M. alba* × *M. polonica*	7.1 a	95.0 a	—
S 711	*M. polonica*, PI 314,386	7.6 a	84.6 a	—
S 710	F_2 *M. alba* × *M. polonica*	8.2 a	95.0 a	—
S 714	*M. officinalis*, var Goldtop[2]	27.9 b	100.0 a	—
S 708	*M. alba*, var Denta, FC 39723	28.9 b	100.0 a	—
S 713	*M. officinalis*, var. Goldtop[3]	39.0 b	100.0 a	—
1970:				
T 592	F_2 *M. alba* × *M. polonica*	0.5 a	32.5 a	3.3 bc
T 590	*M. alba*, var. Denta, FC 39,723	0.9 a	55.0 ab	2.0 a
T 594	*M. polonica*, PI 314,386	1.3 a	59.3 b	3.7 c
T 591	F_2 *M. alba* × *M. polonica*	1.8 a	85.8 bc	3.7 c
T 593	F_3 *M. alba* × *M. polonica*	2.3 a	78.7 c	2.3 ab
T 595	*M. officinalis* var. Goldtop[4]	4.1 b	92.5 c	3.0 abc

[1] Means not followed by the same letter are significantly different from each other—Duncan's Multiple Range Test, 0.05 probability level.

[2] Noncertified Goldtop, Ramy Seed Co. Lot R 1353.

[3] Breeder's Goldtop, FC 38,923.

[4] Breeder's Goldtop, FC 40,001.

TABLE 3.—Observations on root borer larvae in four homozygous sweetclover lines differing in coumarin content and B-glucosidase activity, Lincoln, Nebr., 1969-70[1]

Entries	Geno type	Coumarin content	B-Glucosidase activity	Larvae per plant	Damage score	Infested plants
1969:[2]						
N 741-742	*cucubb*	Low	Low	2.9 a	—[3]	95.0 a
N 744-743	*cucuBB*	Low	High	1.3 a	—	85.0 a
N 746-745	*CuCubb*	High	Low	5.2 b	—	100.0 a
N 748-747	*CuCuBB*	High	High	4.9 b	—	100.0 a
1970:[4]						
N 741	*cucubb*	Low	Low	0.8 a	1.3 a	37.5 a
N 744	*cucuBB*	Low	High	1.0 a	1.2 a	48.6 a
N 746	*CuCubb*	High	Low	3.3 b	2.5 b	83.3 b
N 748	*CuCuBB*	High	High	4.0 b	2.5 b	88.9 b
1971:[4]						
N 742	*cucubb*	Low	Low	3.7 a	3.6 a	100.0 a
N 744	*cucuBB*	Low	High	5.1 a	3.6 a	100.0 a
N 746	*CuCubb*	High	Low	6.7 a	3.7 a	91.7 a
N 747	*CuCuBB*	High	High	7.7 a	3.8 a	94.5 a

[1] Means not followed by the same letter are significantly different from each other—Duncan's Multiple Range Test, 0.05 probability level.

[2] Nonreplicated test, observations made on 20 individual plants of each genotype.

[3] Top growth was killed by frost before a visual rating could be made in 1969.

[4] Randomized complete block design with 12 replications of 3 plants of each genotype in each replication.

[8] HANS, H. and A. J. THORSTEINSON. THE INFLUENCE OF PHYSICAL FACTORS AND HOST PLANT ODOUR ON THE INDUCTION AND TERMINATION OF DISPERSAL FLIGHTS IN "SITONA CYLINDRICOLLIS" FAHR. Ent. Exp. Appl. 4: 165-167. 1961.

[9] GORZ, H. J., F. A. HASKINS, and G. R. MANGLITZ. EFFECTS OF COUMARIN AND RELATED COMPOUNDS ON BLISTER BEETLE FEEDING IN SWEETCLOVER. J. Econ. Ent. 65: 1632-1635. 1972.

[10] SCHAEFFER, G. W., F. A. HASKINS, and H. J. GORZ. GENETIC CONTROL OF COUMARIN BIOSYSTHESIS AND B-GLUCOSIDASE ACTIVITY IN "MELILOTUS ALBA." Biochem. Biophys. Res. Comm. 3: 268-271 1960.

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